•

COLONIZATION OF FABA BEAN *(VIDA FABA)* ROOTS BY DIAZOTROPHS *IN VITRO* AND *IN VIVO* EXPERIMENTS

ABSTRACT

In a laboratory experiment, spermosphere tube was used to investigate the colonization of diazotrophs on plant rootlets of faba bean. Diazotrophs successfully colonized the roots and reduced 2,3,5 tri phenyl tetrazolium chloride (TTC),to TPF . Scanning electron microscopy revealed that *Azospirillum, Bacillus polymyxa* and *Azotobacter chroococcum* were attached to plant rootlets.

A greenhouse experiment was carried out, on faba bean plants (two cultivars) cultivated in a sandy soil, to study the effect of inoculation with *Rhizobium leguminosarum* individually or mixed with *Azospirillum* or *B. polymyxa* or *Azotobacter chroococcum* as co inoculation. Data indicated that co inoculation significantly increased growth parameters *i.e.,* plant height, shoot and root dry weights of two faba bean cultivars , Giza 429 and Giza blanka, nodulation status , microbial activities i.e. total bacterial count, nitrogenase and dehydrogenase activities and seed yield, compared to uninoculated plants.

Key Words*:* Diazotrophs, legumes, *Rhizobium*, Associative N_2-Fixers, Sandy Soil.

INTRODUCTION

The colonization of plant roots by soil-borne or introduced bacteria is a very important step in establishing an effective plant -bacteria interaction. The success of inoculating seeds or seedlings with beneficial bacteria usually depends on the colonization potential of the introduced strains **(Schippers *et al.*, 1987*);* (Weller, 1988).** The beneficial effect of N_2-fixers namely *Azotobacter chroococcum, Azospirillum* and *Bacillus polymyxa* are related not only to their N_2-fixing proficiency, but also with their ability to produce antibacterial and antifungal compounds, growth regulators and siderophores (Pandey and Kumar, 1989). Indirect mechanisms used by PGPR include antibiotic protection against pathogenic bacteria, reduction of iron available to phytopathogens in the rhizosphere, synthesis of fungal cell wall- lysing enzymes, and competition with detrimental microorganisms for sites on plant roots. Direct mechanisms of plant growth by PGPR include the provision of bioavailability phosphorus for plant uptake, nitrogen fixation for plant use, sequestration of iron for plants by siderophores, production of plant hormones like auxins, cytokinins and gibberellins and lowering of plant ethylene levels **(Glick, 1995; Glick *et al*., 1999**

Faba bean *(Vicia faba),* as the most important food legume, has the potential necessity for Egyptian people. The national faba bean area over the last five years is ca. 131.000 hectare with an average productivity of 2. 86 t/ha. Faba bean production in Egypt is sti11limited and fails to face the local increasing consumption of the crop. So, increasing the crop production is one of the major targets of the agricultural policy and can be achieved by both increasing the cultivated area and its productivity.

In the present paper, we compared the effect of inoculation with *Rhizobium leguminosarum* alone or in composite with either *Azotobacter chroococcum, Bacillus polymyxa*, or *Azospirillum brasilense* on plant growth and root -colonization.

M. N.A.Omar*, M.M.El-Shnnawl, S.A.Aboel-Naga ** and M.A.El-Howeity*****
*Dept. of Agric. Microbiology, Soils, Water and Environment Res. Inst., ARC, Giza, Egypt.
** Dept. of Soil Sci., Fac. of Agric., Minufiya University, Shibin ElKom, Egypt.
*** Environmental Studies and Res. Institute (ESRI) , Minufiya University, Sadat city, Egypt.
E-mail: Nabil_omar@excite.com

MATERIALS AND METHODS

Bacterial strains: Strains of *Rhizobium leguminosarum, Azotobacter chroococcum, Bacillus polymyxa* and *Azospirillum brasilense* were obtained from Soils, Water and Environment Res. Inst., ARC, Giza,. These diazotrophs exhibited high acetylene reduction activity (ARA) when grown in N-free **Watanabe and Barraquio** medium **(1979***). Azotobacter* was pre cultured in a modified Ashby's medium **Hegazi** and **Niemela (1976).**The two strains were grown in a nutrient broth liquid medium for 2 days at 30°C, Cultures were then centrifuged at 10000 rpm. for 30 min at 10°C . The sediment was re suspended in 5 ml sterilized 0.8 %KCI (w/v). Bacterial suspension was shacked for 5 min. This material was considered as an inoculum.

Culture Media: Semisolid agar (0.5%) mineral medium was prepared according to **Watanabe and Barraquio (1979).**

Electron microscopy: Scanning electron microscope 'SEM' was used to detect the bacterial colonization (Hinton and Bacon**,1995).**

Seed sterilization: Seeds of faba bean were soaked in a saturated calcium hypochlorite solution for 2 hr under agitation, washed thoroughly in a sterile distilled water, then immersed in 10 % hydrogen peroxide for 20 min. Seeds were germinated in sterilized cultural dishes with two moist filter papers for 2 days at 28°C in the dark. One germinated seed was then sown in a glass tube containing 30 ml of semisolid agar (0.5 %), N-and C-free (WAT) medium.

Experiment I (*ln Vitro*): To investigate the colonization patterns of associative dinitrogen- fixing bacteria on roots, seeds of faba bean were chemically sterilized and sown on to **Watanabe and Barraquia, (1979)** media, free of carbon and nitrogen sources. After 7days from planting, seedlings were inoculated with diazotrophs under aseptic conditions, then 1 m1 of TTC (2,3,5 tripheny1 tetrazolium chloride)was added after 24 hr. to investigate the presence of colonization on roots.

Experiment II (*In Vivo*): Response of faba bean cultivars (“Giza 429" and "Giza' blanka") to inoculation, with *Rhizobium leguminosarum* and co inoculation with Rhizobium plus *Azotobacter chroococcum, Azospirillum or Bacillus polymyxa* under half or full rate of a chemical N- fertilizer, was studded in a pot experiment. Each treatment was performed in four replicates. The experiment was conducted in winter season under greenhouse conditions. Nitrogenase activity (N_2-ase) was measured according to the standard methods of Somasegaran and Hoben (1985). The dehydrogenase activity was carried out according to modified **Thalman (1967).** The Count of bacteria was carried out by direct enumeration according to **Difco Manual (1985**). Five seeds were placed in a potted sandy soil (8 kg/ pot) 30 cm in diameter. The experimental soil was collected from the surface layer (0-30 Cm) of Ismailia Agric. Res. Station .Some physico-chemical properties of the soil are given in Table (1 A &B). Methods of analyses wee according to **Page *et al.(1982).***

Table (1) : Analytical data of the soil used. A. Physical properties

$CaC0_3$%	Particle size distr., %			Texture class
	Sand	Silt	Clay	
1.90	88.59	4.8	6.61	Sandy

B. Chemical Properties

PH*	EC** m mohs/cm	Soluble cations (meq/L)				Soluble anions (meq/L)			
		Ca^{++}	Mg^{++}	K^+	Na+	$C0_3^{--}$	$HC0_3^{-}$	Cl^-	$S0_4^{--}$
7.63	7.20	0.36	0.32	0.13	0.56	---	0.41	0.36	0.61

Organic matter 0.03%

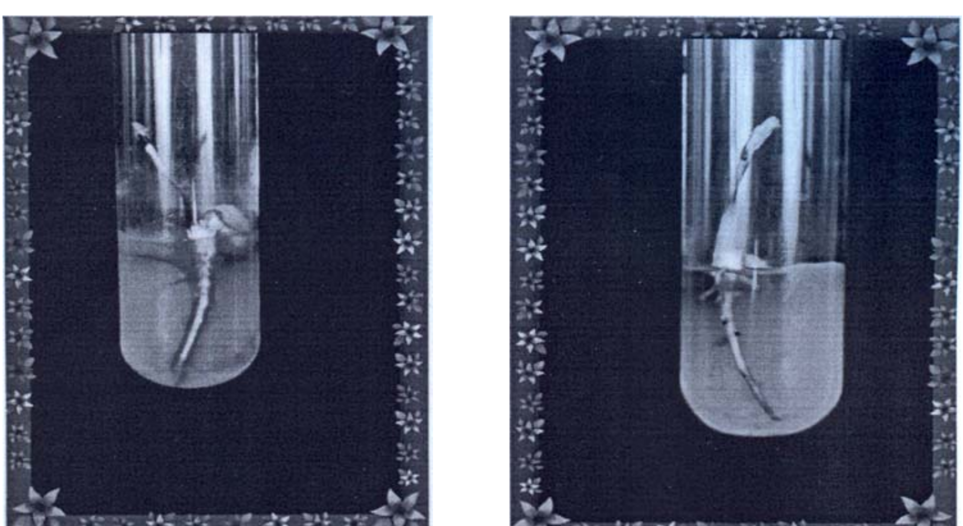
*In the 1 :2.5 soil :water suspension. **In the soil paste extract.

RESULTS AND DISSECTION

Experiment I

Fig. (1) showed that plant roots and reduced TTC to TPF were visible to the eye, and the reducing bacteria could be observed in a red color with the inoculated plants Fig.(1A), while for the uninoculated plants the roots were colorless Fig. (1 B) . The inoculating bacteria were attracted to the plant roots by chemo and air taxis, and then colonized the plant roots, so red color appeared. The colonization sites corresponded mainly to the areas where root mucigel was present. The area around the point of emergence of lateral roots usually showed maximum colonization. **Bilal *et al*.**(1993) found that colonization was mostly present on the root hair, also colonization of the main root surface was also observed. Colonization was probably due to the distribution of receptor structures on the plant root surfaces, relating to sites of greater root exudation. Also, pectinase and exopolysaccharides (EPS) play obvious and important roles in association among the host plant and bacteria.

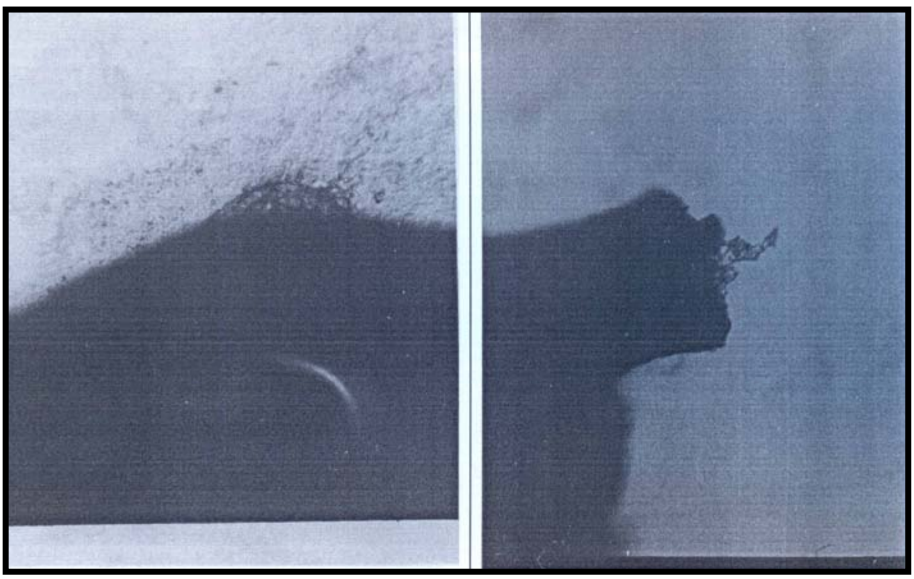
Fig' .(2A) revealed that bacteria colonized plant roots by- light microscopy, where *Azotobacter chroococcum, Azospirillum or Bacillus polymyxa* adsorbed on different plant roots and reduced TTC, while the un-inoculated



A. Inoculated **B. Un- inoculated**

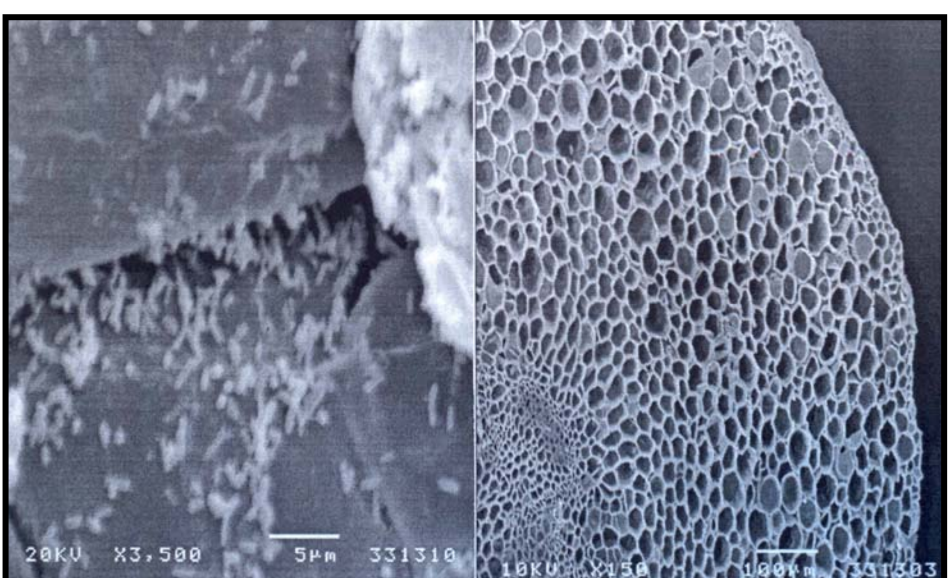
Fig.-1(a and b): Colonization of faba bean roots by associative N_2-fixing bacteria (A) and un inoculated roots (B)of plants grown in spermosphere tubes (stained by 2,3,5triphynyl tetrazolium chloride).

Plants were weekly reduced TTC (Fig. 2B,). Investigation by scannmg electron microscopy 'SEM' showed that successfully *Azospirillum* colonized roots, as compared with the uninoculated plant roots (Figs. 3a and b). Bacterial strains caused root morphology changes (in root hair density, elongation zone length, and total root surface area) (Figs. 1a and 2a). These changes were induced by production of growth - promoting substances i.e. indol acetic acid, gibberellins, cytokinin- like substances. These results agree with those obtained by **Okon, (1982),Lin, *et al*.(1983),Kapulnik *et al*.(1985) and Strzelczyk *et al.* (1994).**



A. Inoculated **B. Un inoculated**

Fig. (2) (A and B): Longitudinal sections of faba bean roots stained by2, 3, 5 triphynyl, Tetrazolium Chloride showing diazotrophs that colonized roots, inoculated (A) and un inoculated (B).



A. Inoculated **B. Un inoculated**

Fig.(3)(a and b): Scanning electron micrographs showing cells of *Azospirillum* which colonized the faba bean roots, inoculated (A) and un inoculated (B).

Experiment II

Plant Growth and Nodulation

Data in Table (2) indicated that inoculation with *Rhizobium leguminosarum* and co-inoculation with *Rh. leguminosarum* plus *Azotobacter chroococcum Azospirillum.* or *Bacillus polymyxa* under half or full rate of mineral N- fertilizer significantly increased plant height as well as shoot and root dry weights, compared to the uninoculated treatment along 60 days after seed sowing. Faba bean "Giza blanka" exhibited an increase of 9.15 % over the "Giza429" cultivars in plant height. Co-inoculation gave 15 % higher plants, compared to that with *Rhizobum* alone by 24 % over control. The highest response of faba bean plants to inoculation was recorded with plants supplemented with the full rate of inorganic nitrogen fertilizer together with the co-inoculation. This enhancement of plant height could be due to a possible N2-fixation and production of plant growth promoting substances by the nitrogen fixing bacteria. These results are in harmony with those obtained by **Plazinski and Rolfe (1985)** and **Fayez *et al*.(1988)** who found that the highest values of all tested parameters were gained when *Azospirillum* was introduced in combination with *Rhizobium.*

Significant increases in shoot and root dry weights were observed when seeds were inoculated with associative diazotrophs combined with a full rate of N-fertilizer. Results in Table (2) exhibited that cultivar "Giza blanka" attained increases of 27.64 and 49.29 % over "Giza 429" in shoot and root dry weights, respectively. Also, co inoculation gave higher increases, of 36.09 and 50.61 %, compared to *Rhizobium* and control, respectively. Integration of co-inoculation with half or full rate of N-fertilizer resulted in significantly higher shoot and root dry weights of both cultivars, compared with un inoculated plants. Increasing the N-fertilizer rate gave increases of 17.69 and 54.67 % of shoot and root dry weights, respectively. Similar results were obtained by **Gallo and Fabbri (1991)** who found that plants inoculated with both *Azospirillum* and *Rhizobium* had a 2- fold dry weights increase as compared with those inoculated with *Rhizobium* alone. Likewise, **Rodelas *et al.(*1999*)*** found that mixed inoculation of *Vida faba* with four different N_2-Fixers namely, *Rhizobium / Azospirillum* and *Rh. / Azotobacter* increased dry weight of plants as compared with inoculation with *Rhizobium* alone. Such increase may be attributed to changes in total N content, concentration and / or distribution of mineral macro- and micronutrients (K+, P, Ca, Mg, Fe, B, Mn, Zn, and Cu).

Table (2): Effect of inoculation with diazotrophs at different levels of N-fertilizer NH_4NO_3) on plant growth of faba bean cultivars.

Bacterial	Nitrogen fertilizer (kg/fed.)	Cultivars: Giza 429 Plant height (cm)	Giza 429 Shoot d.wt. (g/plant)	Giza 429 Root d.wt. (g/plant)	Giza blanka Plant height (cm)	Giza blanka Shoot d.wt. (g / pIant)	Giza blanka Root (g /pIant
Control	10	54.46	8.56	1.58	60.60	12.16	1.58
	20	62.43	10.56	2.07	66.06	14.03	2.78
	mean	58.44	9.56	1.82	63.33	13.09	2.18
Rhizobium leguminosar	10	60.10	9.67	1.64	62.56	13.17	1.98
	20	69.00	11.95	2.45	70.13	15.61	3.23
	mean	64.55	10.81	1.04	66.34	14.39	2.61
Azotobacter chroococcum+ Rh	10	65.50	13.89	1.99	68.56	17.09	2.60
	20	72.53	16.17	2.80	76.00	19.38	4.41
	mean	69.01	15.03	2.39	72.28	18.23	3.51
Bacillus Polymyxa+	10	69.00	14.17	2.23	71.06	17.91	3.20
	20	80.86	15.79	3.20	82.30	19.95	4.77
	mean	74.93	14.98	2.71	76.68	18.93	3.98
Azospirillu brasilense +Rh	10	66.46	12.47	2.03	68.20	16.90	2.71
	20	75.33	14.36	2.78	75.60	18.29	4.61
	mean	70.89	13.41	2.40	71.90	17.59	3.66

Available nitrogen and production of growth promoting substances such as IAA, gibberellins and cytokinine-like substances by PGPR had significant effect on plant growth.

Results recorded in Table (3) showed that the increases in numbers and dry weights of nodules at the two growth periods (50 and 80 day after sowing) were achieved by plants inoculated with *Rh .leguminosarum* and composites of *Rh .leguminosarum* plus *A. chroococcum* or *Azospirillum* or *B. polymyxa* under both half and full rates of chemical N- fertilizer, compared to the uninoculated treatment.

"Giza blanka" exhibited increases of 11.23 and 29.33 % over "Giza 429" in number and dry weights of nodules, respectively. This means that the greatest effect of inoculation was due to the co- inoculation, as compared with *Rhizobium.* The co - inoculation gave higher increases of 28.20, 57.19, 65.59 and 139.52 % over *Rhizobium* alone and the un inoculated plants in nodule numbers and dry weights, respectively. Integration of diastrophic inoculation together with the half rate of nitrogen fertilizer stimulated the nodulation process, while increasing N-fertilizer rate led to a decreased nodulation status. These data are in harmony with the results **of EI-Gizy *et al*. (1999*).*** The nodulation process was obvious at 80 days, compared to 50 days after sowing. The average augmentations in nodule numbers and dry weights of nodules were 85.65 and 129.38 %, respectively. This enhancement of nodulation status could be due to a possible production of plant growth promoting substances by the used inoculant.

Table (3): Effect of inoculation with diazotrophs at different levels of N-fertilizer (NH_4N0_3) on nodulation of faba bean cultivars ..

Bacterial inoculant	Nitrogen fertilizer kg/fed	Cultivars											
		Giza 429						Giza blanka					
		No.nodule / plant			g d.wt.nodules /plant			No.nodule / plant			g d.wt.nodules /plant		
		Days after sowing						Days after sowing					
		80	mean	80	mean	80	mean	80	mean	80	mean	80	mean
Control	10	9.66	17.33	13.49	19.00	39.00	29.00	10.33	20.66	15.49	22.33	28.66	25.49
	20	7.66	15.66	11.66	17.33	31.66	24.49	8.66	19.00	13.83	19.00	24.66	21.83
	mean	8.66	16.49	12.57	18.16	35.33	26.74	9.49	19.83	14.66	20.66	26.66	23.66
Rhizobium leguminosarum (Rh)	10	13.33	22.00	17.66	26.33	44.33	35.33	14.33	22.33	18.33	30.00	52.00	41.00
	20	10.66	19.33	14.99	24.66	41.66	33.16	11.66	20.00	15.83	27.33	45.33	36.33
	mean	11.99	20.66	16.32	25.49	42.99	34.24	12.99	21.16	17.08	28.66	48.66	38.66
Azotobacter chroococcum+Rh	10	15.33	24.00	19.66	32.33	47.33	39.83	17.00	26.00	21.50	34.33	94.66	64.49
	20	11.00	22.33	16.66	25.00	41.00	33.00	14.00	24.00	19.00	28.00	85.33	56.66
	mean	13.16	23.16	18.16	28.66	44.16	36.41	15.50	25.00	20.25	31.16	89.99	60.57
BacillusPorymyxa+Rh	10	16.66	33.66	25.16	34.33	115.00	74.66	18.00	33.66	25.83	37.66	117.66	77.66
	20	12.00	30.33	21.16	27.66	98.00	62.83	15.33	31.00	23.16	32.33	99.66	65.99
	mean	14.33	31.99	23.16	30.99	106.50	68.74	16.66	32.33	24.49	34.99	108.66	71.82
Azospirillum brasilnse+Rh	10	15.00	27.00	21.00	31.00	96.66	63.83	16.00	32.00	24.00	35.00	125.33	80.16
	20	12.66	23.66	18.16	25.66	58.33	41.99	14.33	29.00	21.66	29.66	96.66	63.16
	mean	13.83	25.33	19.58	28.33	77.49	52.91	15.16	30.50	22.83	32.33	110.99	71.66

L.S.D. at alevel of:		5%		1%			5%		1%	
for:		No.	dw.	No.	dw.		No.	dw.	No.	dw.
Faba bean cultivars	A	0.73	9.31	1.11	26.39	AxB	0.81	2.17	1.07	2.87
Bacterial inocula	B	1.37	9.77	2.07	14.80	AxC	0.43	1.16	0.57	1.53
Nitrogen fertilizer	C	0.29	1.69	0.44	2.51	BxC	0.81	2.17	1.07	2.87
						AxBxC	1.14	3.07	1.51	4.07

Total bacterial count

Data in Table (4) showed a slightly increase in the total bacterial counts in the rhizosphere soil of faba bean cultivar "Giza blanka", compared to "Giza 429". Inoculation with *Rh. leguminosarum* and co-inoculation with *Rh. leguminosarum* plus *A. chroococcum* or *Azospirillum* or *B. polymyxa* increased the total bacterial counts in rhizosphere soil of both cultivars, as compared with the uninoculated treatment, where the mean values were 7.62, 7.53, 7.35, 7.01 and 6.78 cfu/ g dry soil with *Rh. leguminosarum* plus *B. polymyxa*, *Rh. leguminosarum* plus *Azospirillum* , *Rh. leguminosarum* plus *A. chroococcum* , *Rhizobium leguminosarum* and control for "Giza 429" and "Giza blanka", respectively. The co-inoculation with *Rh. leguminosarum* plus *Bacillus polymyxa* was more pronounced when gave higher increase above *Rh. leguminosarum* plus *Azospirillum* , *Rh. leguminosarum* plus *A. chroococcum*, *Rhizobium leguminosarum* alone, control in the descending order, 7.74, 7.67, 7.61,7.14 and 6.93 cfu / g dry soil, respectively. The increases were 6.99, 10.61 % and 7.46 and 10.67 % over *Rhizobium* and control of faba bean "Giza 429" and "Giza blanka", respectively. The obtained results declared that the total bacterial count increased with increasing N-fertilizer rates particularly in presence of the recommended full rate compared to the half rate. These data are in harmony with those obtained by **EI-Gizy *et al.*(1 999),** who stated that inoculation of *Phaseolus vulgaris* with *Azospirillum* increased total bacterial count in rhizosphere area.

Enzyme Activities

The applied treatments in general led to significant increases in the activity of dehydrogenase (DHA) in the rhizosphere soil cultivated with faba bean cultivars (Table4). These results were more obvious when co-inoculation was used, *Rh. leguminosarum* plus *Bacillus polymyxa* for both cultivars, when they gave 5172.03 and 5789.97 ul H / g dry soil! 24 hr. for "Giza 429" and "Giza blanka", respectively. Such increases represent 41.01 and 51.79 % and 35.96 and 49.11 % over *Rh. leguminosarum* alone and control for "Giza 429" and "Giza blanka", respectively. No significant differences were obtained between plant cultivars in DHA. Increasing the N-fertilizer rate led to increase DHA the growth period, but not significantly between the N fertilizer doses. The full recommended N rate gave higher values, as compared with the half dose. On the other hand, a comparison between the different inoculant in concern of their stimulatory action on DHA in the rhizosphere soil cultivated with the faba bean plants, showed the following descending order:

Rh. leguminosarum plus *B. polymyxa*> *Rh. leguminosarum* plus *Azospirillum* > *Rh. leguminosarum* plus *A. chroococcum*> *Rh. Leguminosarum.*

Data obtained for N_2-ase activity showed that co-inoculation gave considerably higher values of such enzyme, compared to those obtained with *Rh. Leguminosarum* alone. The highest N_2-ase activity levels were recorded at the 60th day of cultivation for cultivars. When comparing the effect of the different dinitrogen-fixers on N_2-ase activity in the rhizosphere of faba bean plants, *Rh. Leguminosarum* plus *Bacillus polymyxa* were superior in enhancement of N_2-ase activity, when they gave 78.18 and 130.73 n mol C_ZH_4/ hr/ g dry weight of plant for "Giza 429" and "Giza blanka", respectively.

Table (4): Effect of inoculation with N_2-fixing bacteria and application of different rates of N-fertilizer (NH_4 NO_3) on enzyme activities in the rhizosphere of faba bean cultivars.

Bacterial inoculant	Nitrogen fertilizer (kgN/fed.)	Total count'		DHA activity"		N_2 ase activity'"	
		Giza 429	Giza blanka	Giza 429	Giza blank	Giza 429	Giza blank
Control	10	7.07	7.05	3258.08	3482.10	28.14	52.90
	20	7.13	7.12	3674.55	4018.85	15.66	43.03
	mean	7.10	7.08	3466.31	3750.47	21.90	47.96
Rhizobium leguminosarum +	10	7.18	7.13	3423.46	3599.37	43.97	79.01
	20	7.25	7.22	3851.96	4465.39	24.39	62.85
	mean	7.21	7.17	3637.71	4032.38	35.18	70.93
Azotobacter chroococcum+(Rh)	10	7.39	7.66	4573.64	4799.17	91.52	120.69
	20	7.59	7.78	4954.03	5860.64	35.39	84.42
	mean	7.49	7.72	4763.83	5329.91	63.45	102.55
Bacillus Polymyxa +(Rh)	10	7.59	7.76	4874.34	5287.80	107.12	157.17
	20	7.90	7.94	5469.73	6292.14	49.24	104.29
	mean	7.74	7.85	5172.03	5789.97	78.18	130.73
Azospirillum brasilense +(Rh)	10	7.40	7.68	4654.83	5066.79	93.15	127.44
	20	7.84	7.85	5218.64	6104.21	46.68	94.95
	mean	7.62	7.76	4936.73	5585.50	69.91	111.19

* Total bacterial count: Log number of cfu / g soil.

** DHA: Dehydrogenase activity in rhizosphere soil (ul H / g dry soill 24 hr).

*** N_2-ase activity: Nitrogenase activity in rhizosphere plant roots (n mol $C_2H_4$1 hr / g dry weight of plant).

Seed and Straw Yields of Faba Bean

Results of Table (5) indicated that inoculation with Rhizobium l or other N2-fixers. Significantly increased both grain and straw yields as compared to the control of both cultivars. Such results were more evident, when the co-inoculation was used, as compared with the control and Rhizobium a lone. The increases in grain yield being 36.27& 62.26 % and 32.79& 52.03 % over Rhizobium alone and uninoculated (control) treatments for" Giza 429" and "Giza blanka", respectively. Also, the increases in straw yield were 16.22& 37.70 % and 24.20& 38.37 % over Rhizobium alone and the uninoculated (control) treatments for "Giza 429" and "Giza blanka", respectively.

It was clear that the cultivar "Giza blanka "exhibited an increase of27.15 % higher than the cultivar Giza 429. Nitrogen application significantly increased seed and straw yields. It could be observed that there was a gradual response in grain and straw yield of faba bean cultivars owing to the increase in N- levels. Promotion of crop yield with raising the nitrogen supply might be due to the rise in the dry weight of vegetative organs, which could be considered as a criterion for the photosynthetic efficiency of the plant. On the other hand, a comparison between the different inoculant for their role in increasing the crop yield displayed the following descending order:

Rh + *B. polymyxa* > Rh + *A. chroococcum* > Rh+ *Azospirillum* > *Rhizobium* alone, when they gave 37.22, 34.06, 31.62 and 25.83, and 28.70, 26.48, 25.97 and 19.85 g grain / plant for faba bean "Giza blanka" and "Giza 429", respectively. Such increases might be attributed to the available nitrogen supplemented by the inoculating microorganisms, as a result of encouraging N_2-fixation activity .The production of growth substances such as AAI, gibberellins and cytokininie- like substances which stimulate the yield of faba bean. These results are in agreement with those obtained by **Srinivasan *et al.(1996),*** **EI-Gizy *et al.*(1999), Rodelas *et al.* (1999), Omar *et al.*(2000), Massoud (2001)** and **Mekhemer (2001),** who found that co-inoculation of faba bean and pea seeds with *Azotobacter, Azospirillum* and *Bacillus polymyxa* significantly increased grain and straw yields.

Table (5): Effect of inoculation with diazotrophs and application of different levels of N- fertilizer (NH_4N0_3) on yield of faba bean cultivars.

Bacterial inoculant	Nitrogen fertilizer level (kg/fed.)	Faba bean cultivars			
		Giza 429		Giza blanka	
		Yield, g/ plant			
		Seed	Straw	Seed	Straw
Control	10	15.21	30.57	20.88	39.70
	20	18.14	32.62	24.24	41.82
	mean	16.67	31.59	22.56	40.76
Rhizobium leguminosarum (Rh)	10	18.00	36.33	25.01	43.61
	20	21.70	38.53	26.66	47.22
	mean	19.85	37.43	25.83	45.41
Azotobacter chroococcum+Rh	10	25.05	42.06	31.78	53.02
	20	27.92	44.59	36.34	57.91
	mean	26.48	43.59	34.06	55.46
Bacillus Polymyxa+Rh	10	26.15	43.69	34.74	55.75
	20	31.26	47.00	39.91	64.18
	mean	41.78	45.34	37.22	59.96
Azospirillum brasilnse+Rh	10	24.10	40.42	29.20	50.68
	20	27.84	42.75	34.05	56.90
	mean	25.97	41.58	31.62	53.79

L.S.D. at level of:		5%		1%			5%		1%	
for:		G.	St.	G.	St.		G.	St.	G.	St.
Faba bean cultivars	A	1.21	1.83	2.31	3.50	AxB	0.77	1.03	1.11	1.48
Bacterial inocula	B	2.26	3.43	4.32	6.55	AxC	0.41	0.55	0.59	0.79
Nitrogen fertilizer rate	C	0.29	0.39	0.42	0.56	BxC	0.77	1.03	1.11	1.48
						AxBxC	1.10	1.46	1.57	2.10

REFERENCES

Bilal, R; G.Rasul; M.Arshad and K.A.Malik (1993) Attachment, colonization and proliferation of *Azospirillum brasilense* and *Enterobacter spp.* on root surface of grasses. World J. Microbiol & and Biotech. ,9:63-69.

Difco Manual (1985). Dehydrated culture media and reagents for microbiology .1OthEd.Difco Laboratories Defroit Michigan 4823, USA:487-623.

El-Gizy,S.M.; A.M.shalaby and M.M.Hanna (1999). Effect of *Azospirillum* inoculation and nitrogen fertilization on the yield of some cultivars of Phasealus vulgaris. J.Agric. Mansoura Univ.,24(8): 4201-4210.

Fayez, M.;Nadia F.Emam and H.E.Makboul (1988). Interaction between *Azospirillum brasilense* and *Rhizobium leguminosarum* bv. Viceae and their influence on nodulation and growth of broad bean *(Vicia faba*). Proc. 2nd. Conf. Agric. Develop. Res. , Ain Shams University.

Gallo, D.M. and P.Fabbri (1991). Effect of organic matter on chick pea inoculated with *Azospirillum brasilense* and *Rhizobium leguminosarum* bv. Ciceri. Plant and Soil, 137: 171-175.

Glick B.R. (1995).The enhancement of plant growth by free -living bacteria. Can .j. Microbial.41:109-117.

Glick B.R.; C.L.Patten; G.Holguin and D.M. Penrose (1999). Biochemical and genetic mechanisms used by plant growth -promoting bacteria . Imperial College Press, London, UK.

Hegazi,N.A.and S.Niemela(l976) .A note of the estimation of *Azotobacter* densities by memberane filter technique lApl.Bacteriol.,41 :311.

Hinton, D.M. and C.W.Bacon(1995). *Enterobacter cloacae* IS an endophytic symbiont of com. Mycopathologia, 129: 117-125.

Kapulnik,Y.R.; Y. Okon, and Y.Henis (1985). Changes in root morphology of wheat caused by *Azospirillum* inoculation. Can. lMicrobiol.,31:881-887

Lin,W.;Okon, Y. and R.W. Hardy (1983). Enhanced microbial uptake by *Zea mays* and sorghum roots inoculated with *Azospirillum brasilense.* Appl. Environ. Microbiol.,45:1775-1779.

Massoud, A.M. (2001). Effect of molybdenum on nitrogen availability in soil and its metabolism in plant. Ph.D. Dept., Soil S ci., F ac., A gric., M inufia U niv., Egypt.

Mekhemar, G.A.A. (2001). Response of faba bean plants to co-inoculation with *Rhizobium leguminosarum* and *Bacillus megaterum* under different levels of phosphate fertilization in newly reclaimed lands. J. Agric. Sci. Mansoura Univ. ,26(12): 8129-8140.

Okon,Y.(1982). *Azospirillum:Physiology,properties,mode* of association with roots and its application for the benefit of cereal and forage grass crops .Israel J.Bot.,31: 214-220.

Omar, M.N.A.; A.T.Mostafa and A.S. Ahmed (2000). Concentrated vinasse as a Novel diazotrophs growth medium (Biovinasse Inoculant) and soil conditioner improve f aba bean yield under dripping irrigation. Proceedings of the Tenth Microbiology Conf., Cairo, Egypt, 11-14 Nov., pp. 100-109.

Page,A.L.;R.H.Miller and D.R.Keeney (1982). Methods of Soil Analysis, Part 2 : Chemical and Microbiological Properties, 2nd ed Amer. Soc. Agron. Inc. &Soil Sci. Soc. Amer., Inc.Madison, Wisconsin, USA.

Pandey, A. and S.Kumar (1989). Potential of Azotobacters and Azospirilla as biofertilizers for upland agriculture: A Review. J. Sci., and Industrial Res., 48: (3):134-144 .

Plazinski, J. and B.G. Rolfe (1985) .*Azospirillum- Rhizobium* interaction leading to aplant growth stimulation without nodule formation. Con. J. Microbial 31: 1026-1030

Rodelas, B.;J. Gonzalz-Lopez;C.Pozo; V.Salmeron and M.V.Martinez- Toledo (1999). Response of faba bean *(Vicia faba*) to combined inoculation with *Azotobacter* and *Rhizobium leguminosarum* bV.viceae. Applied soil Ecology 12,51-59.

Schippers B; AW. Bakker; P.Bakker (1987). Interaction of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Annu. Rev. Phytopathol, 25:339-358.

Somasegaran,P.and ,H.G.Hoben (1985). Methods in *legume-Rhizoium* technology , NIFTAL. Project and MIRCEN, Department of Agronomy and Soil Science, College of Tropical Agriculture and Human Resourse, Univ. Hawaii, USA. :320-327.

Srinivasan, M.;D.J.Petersen and F.B.Holl (1996). Influence of indoleacetic - acid - producing *Bacillus* isolates on the nodulation of *Phaseolus vulgaris* by *Rhizobium etli* under gnotobiotic conditions. Can. J. Microbiol.,42: 1006-1006.

Strzelczyk,E.;M.Kampert and C.Y.Li (1994). Cytokinin-like substances and ethylene production by *Azospirillum* in media with different carbon sources .Microbiol.Res., 149 : 1-6.

Thalmann,A.(1967). Uber die microbiello aktiviatat merkma1en einiger acherboden unter besonderer beruksi chtigung der dehydrognase activiatat (T.T.C. Reduktion).Biss, Gieben.Ph.D.Thesis, W. Germany.

Watanabe,I.and W.I.Barraquio (1979). Low levels of fixed nitrogen required for isolation 0 f free- living N_2- fixing organisms from rice roots. Nature, 277: 565- 566.

Weller, D.M.(1988). Biological control of soilborne plant pathogens **III** the rhizosphere with bacteria. Annu. Rev. Phytopathol.,26: 379- 407.

تكوين المستعمرات البكتيريه علي جدور نباتات الفول البلدي في المعمل والصوبه بالبكتيريا المثبته للازوت الجوي

محمد نبيل عبد المجيد عمر [1] ماهر الشناوي [2] صلاح ابو النجا [2] محمد الحويطي[3]

1 -معهد بحوث الاراضي والمياه والبيئه – مركز البحوث الزراعيه – الجيزه
2- كليه الزراعه شبين الكوم – جامعه المنوفيه – المنوفيه
3- معهد الدراسات البيئيه -جامعه المنوفيه -مدينه السادات

الملخص

تم دراسه تكوين المستعمرات البكتيريه علي جدور نباتات الفول البلدي معمليا في انابيب الزراعه (سبيرموسفير موديل) . اثبتت بعض انواع من البكتيريه المثبته للازوت الجوي مثل الازوتوباكتر كروكوكوم والازوسبيريللم برازيلنيز و الباسلس بوليمكسا نجاح عمليه تكوين هده المستعمرات البكتيريه باستخدام الميكروسكوب الاكتروني واختزال المركب الكيماوي عديم اللون 2و3و5 تراي فينايل تترازوليم كلورايد(TTC) الي ماده تراي فورمازان دات اللون الاحمر عند قياس اللون علي جهاز Spectrophotometer طول موجه 485 نانوميتر.
اقيمت تجربه اصص في الصوبه علي نباتات الفول البلدي باستخدام صنفين هما جيزه 429 و جيزه بلانكا في ارض رمليه لدراسه تأثير التلقيح البكتيري بالريزوبيا منفرده او مختلطه مع الازوسبيريللم و الازوتوباكتر و الباسلس مع تركيزات مختلفه من التسميد الازوتي المعدني. اوضحت النتائج ان التلقيح المختلط ادي الي زياده معنويه في النمو الخضري للفول البلدي (طول النبات – الوزن الجاف للسيقان والجدور -حاله العقد الجدريه) وكدلك النشاط الانزيمي مثل النيتروجينيز والديهيدروجينيز بالاضافه الي محصول الحبوب .